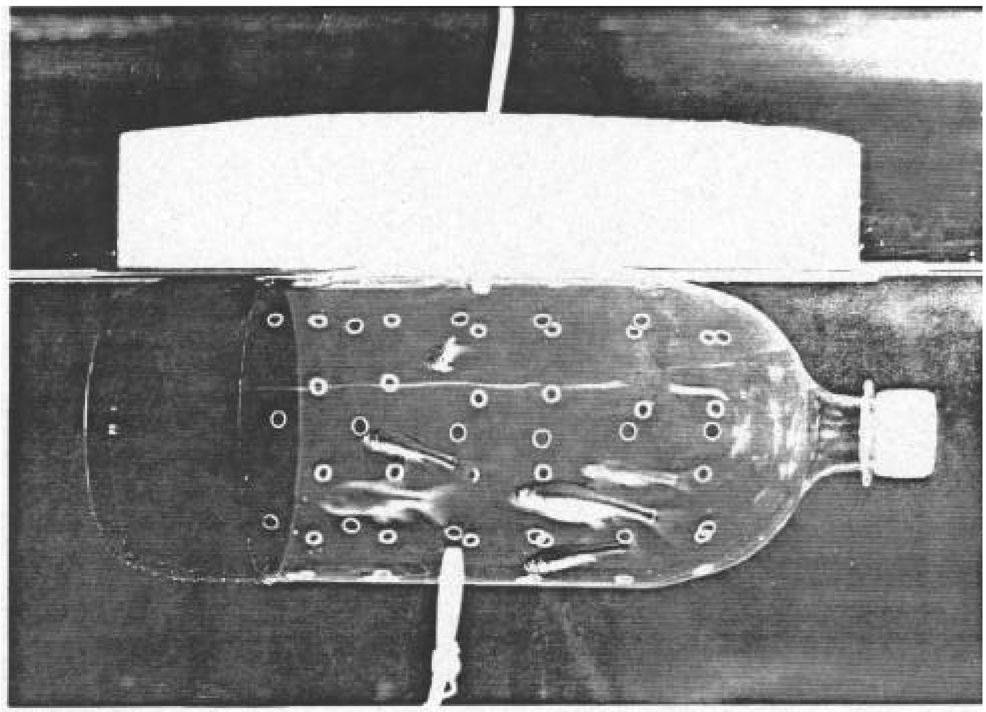
SECTION 2
STREAM SENTINEL OPERATIONAL GUIDE



City of Fort Worth
Department of Environmental Management
5000 Martin Luther King Freeway
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USEPA Section 104(b)(3) Grant Project

FOR YOUR INFORMATION

During the discussions in the accompanying video of alternative biological monitoring methods, terms such as "biomonitoring", "bioassessment", "biosurvey", and "biological monitoring" are used somewhat interchangeably and may create some confusion or misconceptions for persons familiar with the "biomonitoring" tests required in many municipal and industrial wastewater discharge permits. For example, many permitting authorities have historically used the term "biomonitoring" to refer only to 'Whole Effluent Toxicity testing (WET)". Please be aware that "bio-monitoring" in this video applies to all biological monitoring methods, including biosurveys that sample the water's natural life and the biotoxicity screening introduced in these videos. The videos are not intended to imply in any way that "biological monitoring" of storm drain discharges is limited to WET testing methods.

The stream sentinel is intended to monitor dry weather flows in urban storm drainages.

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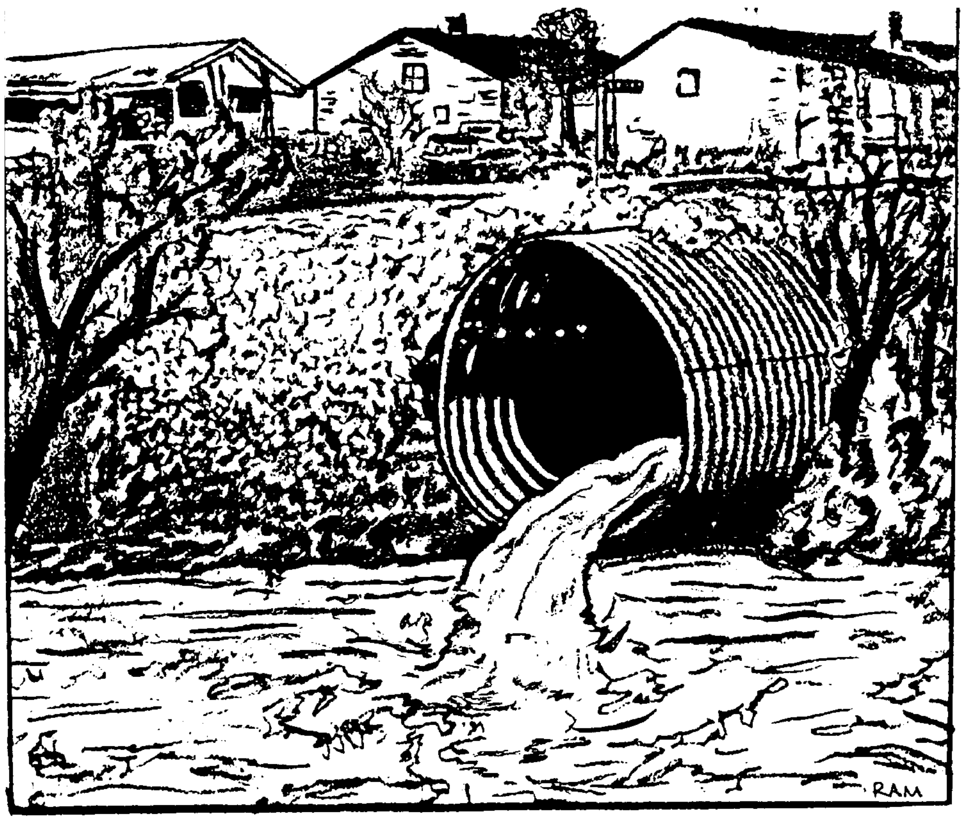
INTRODUCTION



This booklet provides enough "how to" information so that a typical municipal agency can establish a network of water quality monitoring stations in its storm drainage system. These stations will monitor water quality on a 24 hour basis and provide data results which can be recorded at stream side. These stations, which are called stream sentinels, are inexpensive to construct/operate and don't require the exclusive use of highly trained technical staff to evaluate water quality.

The technology is simple in that a test organism (fathead minnow) is exposed to flows in an urban storm drainage creek or outfall for multiple days. If the test minnows die, it is likely that a pollutant was present at some time since the minnows were last observed. Additional screening can be conducted to determine if there is some constancy or repetition to the toxicity occurrence and additional sentinel units may be placed throughout the storm drainage to isolate the source of the toxic flows during dry weather. Corrective actions can be then initiated.

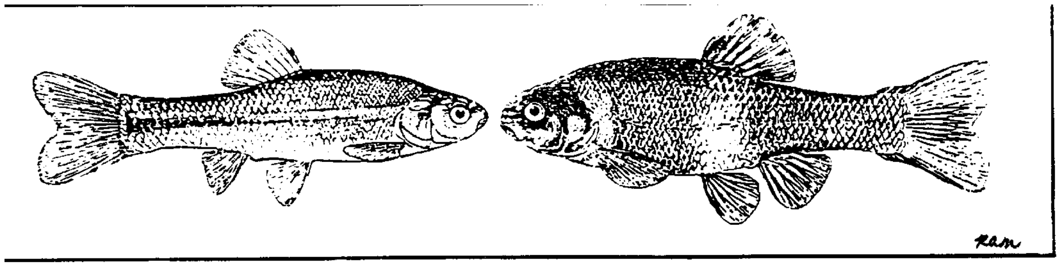
The technology is inexpensive since all of the equipment and supplies are readily available and can be easily assembled by municipal staff.

The most challenging element of the entire program is in maintenance of the minnows at the municipal field office. A staff member (or private contractor) should be selected who has an aptitude for raising tropical fish. Maintenance of the minnows is much less complicated if the municipal staff is on friendly terms with the personnel from a local aquarium, tropical fish store, zoo aquarium or local university biology department.

Enough detail is provided in the following pages to provide a municipality with adequate information needed to operate a stream sentinel monitoring program. Should additional information be needed, staff members who participated in this project are listed in Acknowledgments. They may be contacted through the City of Fort Worth Department of Environmental Management at (817)871-545 1.

This booklet was taken, in its entirety, from "Biotoxicity Testing: Screening methods for Monitoring Wet and Dry Weather Discharges From Municipal Storm Drain Systems", which was written by the City of Fort Worth Department of Environmental Management employees. This project was supported by a Section 104(b)(3) grant from the United States Environmental Protection Agency.

THE MINNOWS



DISTRIBUTION, TAXONOMY AND MORPHOLOGY

The test organism used in this study is the fathead minnow, *Pimephales promelas.* It is widely distributed across North America with a natural range from the RockyMountains to the Appalachian Mountains, Northwestern Canada southward to the Gulf States. They have been introduced to the east and west coasts. Due to its popularity as a bait minnow, fishermen have expanded the fatheads range to the point that their original range is now difficult to determine.

There are four recognized species of *Pimephales.* These are: *Pimephales promelas,* the fathead minnow; *Pimephales notatus,* the blunthead minnow; *Pimephales vigilax,* the buuhead minnow; *Pimephales tenellus,* the slim rminnow. The fathead is usually the most abundant species in its preferred habitat. Also, some geographic variations have been noted in *promelas* and several populations have been designated as sub specifically different. However, The American Fisheries Society (1980) recognize only one species.

Fatheads are most abundant in small waterways and ponds. They prefer slow currents and boggy, mud-bottomed ponds. They are rare or totally absent in large, deep impoundments and high gradient streams.

Mature fatheads are 1.5 to 3.5 inches in length and ofivaceous. Scales are moderate in size and cycloid. The mouth is terminal and oblique. The lateral line is nearly complete although it is less complete in many northern populations. Dorsal fin is distinctly rounded and divided by a dark, horizontal bar. A dark spot is sometimes present in front of the dorsal on males. Spawning males with a saddle shaped marking on the body beneath the dorsal fin. These males also turn nearly black develop a thick pad on the back, from the snout to the second dorsal ray and nuptial tubercles on the snout. The tubercles form in three main rows with a few usually appearing in the lower jaw. Mature females have a distinct ovipositor.

THE MINNOWS - SOURCES

A constant supply of healthy minnows is crucial to the success of a biomonitoring program. There are two ways to assure a continual supply:

(1) Purchase your stock

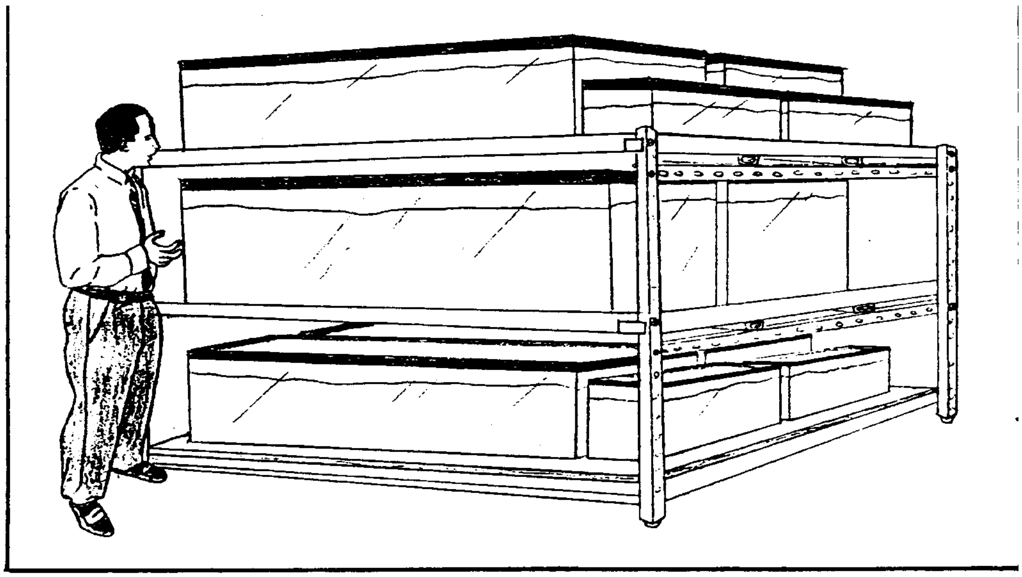
This method involves the construction of a large holding tank or series of holding tanks to temporarily store the minnows until they are needed. With this system, the minnows are purchased as adults and replenished when numbers become low.

Most commercial minnow farms keep a good supply of fathead adults in their inventory. Prices range from $2.00 to $5.00 per hundred depending upon the total quantity ordered. However, minnows brought in from this type of commercial operation can carry a multitude of diseases due to the crowded conditions common to these farms. Adult minnows seined or trapped from local waters may be used and have an advantage over those from other sources as they are already acclimated to local water quality parameters. All minnows brought in from outside sources must be quarantined for a minimum of 14 days and usually given a prophylactic course of antibiotics to safeguard against the introduction of infections to the laboratory aquariums. Once the quarantined minnows are deemed healthy and free of all diseases, they may be used in the field or as breeders.

(2) Raise your stock

If adult fatheads are not easily available, a breeding program must be established to assure a continual supply of specimens. As healthy test organisms are vital, breeding stock should come from a well established laboratory culture. Many local colleges and universities utilize fatheads in toxicity testing and are usually happy to supply someone with a few fish to start a breeding program. The USEPA Environmental Research Laboratory in Duluth, Minnesota offers fathead embryos and occasionally juveniles.

STAFF and SPACE REQUIREMENTS



STAFF REQUIREMENTS

Staff requirements for a biomonitoring program will vary depending upon the extent of the program. However, a minimum of one staff member will be needed for even the smallest program commitment. Medium and large municipalities will need additional staff hours to maintain the aquariums, conduct weekend work such as feeding or monitoring and to expand the monitoring program to suit the municipality's needs.

The number of staff hours (or full time equivalent positions, F.T.E.) will depend upon a wide range of site specific conditions found in municipalities. These conditions include the geographical size of the city, number of storm drain outfalls or suitable drainage channels, population, number of rain days, degree of pollutant problems in the storm drainage system, duration of frozen surface water season, etc. In consideration of these variables, it is recommended that a minimal program is established and then expanded if needed, after the municipality becomes familiar with its monitoring needs. For medium or larger municipalities, two full time equivalent positions would be needed to establish and operate a monitoring program. Cities could use full or part time assistance from enlisting city staff, temporary employees or volunteers to fulfill the 2 F.T.E. minimal requirements (2,080 hours X 2 = 4,160).

It should be noted that if full time permanent staff are hired to work this program (preferred choice), then these staff could be cross utilized for special projects during temporary breaks or suspensions in the monitoring effort. This would promote flexibility while providing some measure of "bum out" proofing of employees. If the municipality has adequate numbers of staff, the field work could be rotated through different programs. However, aquarium maintenance must be restricted to only one or two staff members who are knowledgeable in the care and feeding of minnows.

There are 3 basic staff functions. These include:

(1)Placing and replacing stream sentinel units in the field and monitoring the results.
(2)Maintaining the aquarium operations. This will be more labor intensive if the municipality elects to establish a breeding program as opposed to maintaining purchased stock.
(3)Maintaining records and analyzing data as a result of the monitoring program.

For monitoring, a minimum of two people are needed. One person is used to place sentinel units and monitor them on a daily basis. A second staff member will be needed to perform weekend site checks, place additional sentinel units, and perform aquarium maintenance. The second member of the monitoring staff can be a part-time employee.

BUILDING REQUIREMENTS

The space needed for aquariums is dependent upon the size and overall scope of the program chosen. As a general rule, more space will be needed for a breeding program than a program that simply holds adult minnows until needed. The Fort Worth program uses 8 rearing tanks that range from 40 to 75 gallons each and 4 spawning tanks of 10 gallons each. It takes at least a 10' X 15' space for this set-up.

Any space chosen must have a floor capable of supporting the significant weight of a large aquarium set-up. The floor should be concrete, tile or some other type of waterproof material. Also, the floor is often wet so rubber mats should be utilized to minimize the chance of slips or accidental contact with electrical devices. Ground fault interrupters should be installed on all electrical outlets in the aquarium area. The area must also have adequate lighting to perform all operations. The use of extension cords should be minimized and any electrical service or cords should be wall mounted. Any in-floor electrical outlets must be removed.

Any aquarium area must have easy access to a water source and a large sink will be needed to clean equipment such as filters. A floor drain is a nice option to have in any aquarium area as spills are unavoidable and aquariums occasionally leak or break.

Ideally, the space chosen would be a room on its own where all of the above features could be incorporated along with a climate or temperature control system. However, the Fort Worth site was created by partitioning off a portion of an office.

TYPES OF AQUARIUM SYSTEMS

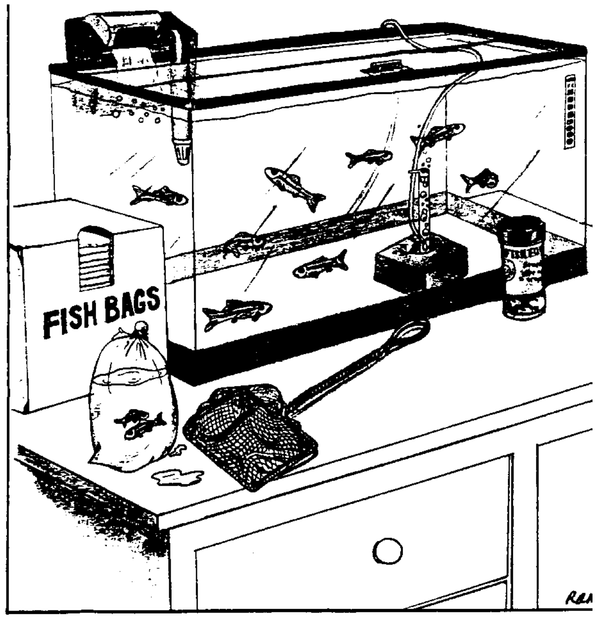
There are three types of aquarium systems commonly employed in the maintenance or breeding of fish. These are- constant flow, recirculating and static.

The constant flow is a system where fresh water constantly trickles into the aquariums, flowing out through an overflow pipe. The water source, often tap water, is dechlorinated and chemically treated as needed with a series of in-line injectors before being pumped to the aquariums. The overflow water is typically discharged to the sanitary sewer system.

The recirculating system is similar to the constant flow except that the overflow water is filtered and then reintroduced into the system. For fast growth rates, limited disease and minimal maintenance, the constant flow and recirculating systems excel and should be considered for any long term studies. However, these are the most expensive systems and can be complex to set up. For short term or limited studies of one to three years, a static system is the best choice.

A static system is the normal " stand alone " aquarium set-up consisting of a tank, air pump and filter. This is the system that was used in the Fort Worth study and specific components will be discussed in detail throughout this guide.

The space needed for aquariums is dependent upon the total number of minnows needed for the study. Approximately 300 fatheads can be reared to maturity in a 40 gallon aquarium in 90 to 120 days. Numerous rearing tanks are needed to accept juveniles of differing ages and sizes, thus assuring a continuous supply of mature specimens for placement in the stream. The actual number of rearing tanks will depend upon the total number of minnows needed to complete the study. A program using six rearing tanks will supply 75 to 100 minnows per week under ideal conditions.



EQUIPMENT SOURCES

The best source of new aquariums and aquarium supplies is a large, wholesale pet supply distributor. These distributors are easily located through the Yellow Pages or a local business directory. Also, the manager of a well established pet shop can offer leads on wholesaler suppliers in the area most likely to give the best deals. Any supplier worth dealing with will offer a products catalogue for price comparisons and bid selections.

Retail aquarium/pet shops can order all of the equipment and supplies needed to set-up a fathead breeding laboratory but very few will actually have all of these items in stock. Prices in one of these shops will be considerably higher than wholesale for new equipment. Most retail dealers have contacts for used equipment and will often sell used shop equipment at a substantial discount off retail.

Other sources of new and used equipment include discount house close-out sales, auctions, municipal zoos, nature centers, schools and fish hatcheries. Limit used equipment purchases to tanks, covers, tubing, etc. Anything mechanical, such as air pumps or filters, should be purchased new as they are subject to the most wear and tear of all the equipment. Their condition and proper operation are critical to the minnows survival.

Keep in mind that all used equipment must be cleaned *and* sterilized using the appropriate sterilization solutions for the materials involved.

AQUARIUMS

A wide variety of aquarium sizes and types are utilized in a typical minnow breeding program. The mature, spawning pairs will each need a tank of their own. Ten gallon aquariums were used in this study and they worked well. Others have successfully used 20 gallon aquariums with plastic or stainless steel dividers for housing multiple pairs.

Rearing tanks should be as large as space and budget allow. Many minnow breeding programs **place** newly hatched fathead larvae into 20 gallon (24"X 12"X 16") aquariums for the first 15 to 30 days. After this initial growth period, they are transferred to larger rearing tanks.

The 40 gallon breeder aquarium is ideal for rearing minnows where space is critical. This compact aquarium is only 36"L X 18"W X 16"H yet has a very large surface area, comparable to tanks much larger. This is, typically, the most commonly used tank in most fish hatcheries.

Rearing tanks need not be all glass. Fiberglass and a variety of plastics are being utilized by tank manufacturers and these alternatives are often much less expensive than glass aquariums in sizes of 75 gallons or more. Do not use any tanks with metal on them, where the metal contacts the water, for scientific studies. Metallic buildups in the aquarium water will affect the minnows overall condition and could influence the study results.

If possible, keep all aquariums and tanks covered at all times. Covers help to keep evaporation and the subsequent mineral buildup in the aquarium water at a minimum. Dust and other air bom contaminants can also cause problems and should be kept to a minimum. Glass is the preferred cover material as it is easy to clean and sterilize.

AIR PUMPS

After the aquafiums are picked out, the next decision involves the air delivery system. There are two basic methods of getting air to the aquafiums. The first utilizes a large, centrally located compressor which supplies air to all tanks in the system. PVC pipe is used to transport air from the compressor to the tank area and small plastic or brass valves, inserted into the PVC, deliver air to each individual aquarium.

The second system utilizes small, individual pumps, commonly known as " vibrator " pumps in the aquarium industry. Each pump supplies air to one or two aquariums (Fig. I).

Naturally, there are advantages and disadvantages to each system. The compressor system is easy to set up and maintain, plus it is cheap to operate over an extended period of time. Unfortunately, air from compressors is rarely clean enough for aquarium use and must be well filtered for oil and carbon. Also, when the compressor eventually breaks down, and it will, it must be repaired immediately, no matter what day it is or what time it is. Dissolved oxygen levels can drop alarmingly fast when the air supply is interrupted.

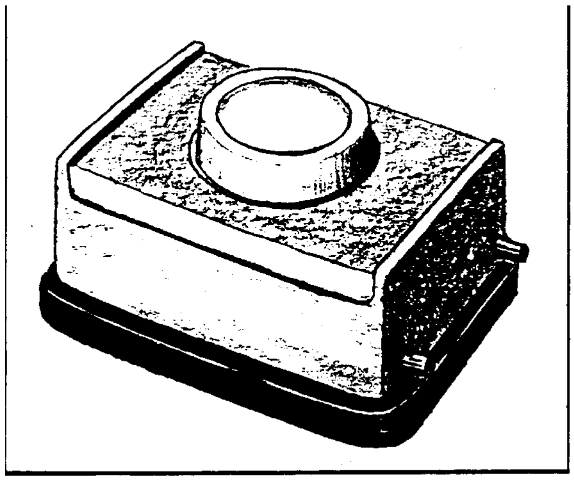


Fig. I - Typical vibrator pump with dual air outlets

The second system, as used in this study, utilizes small, individual air pumps. This is the most dependable air delivery system in that if a pump goes out in the middle of the night, only one tank is in danger of being lost. While a single pump can usually handle several aquariums, it is best to have one pump per tank for the reasons described above. Parts and extra diaphragms for these smafl pumps are readily available and the pumps themselves are easily rebuilt in only minutes. Typically, the rubber diaphragm in a vibrator pump will last 18 to 24 months.

To complete the air delivery system additional purchases of valves, tubing and airstones will be necessary. A selection of brass 2-way and 3-way valves is needed to divide air flows and regulate pressure. Clear, airline tubing is standardized to fit all aquarium equipment and is available in inexpensive, bulk rolls.

Airstones and air diffusers are available in dozens of shapes and sizes. They are also made from a multitude of materials. Most airstones are easily broken and often clog within few months so three times as many should be purchased as is actually needed. Any airstone considered should emit fine bubbles with a minimum of air pressure. The aquarium dealer/representative can help choose the best airstone for a specific need.

FILTERS

Three types of filters are used in the Fort Worth facility. A sponge style hatchery filter is used in all tanks (Fig. 2). The sponge does a good job of filtering out particulate matter , plus, nitrif)ing bacteria will colonize the filter within 96 hours making the sponge an effective biological filter as well. This is the only style of filter that will not harm minnows during the juvenile stages. Sponge filters should be rinsed weekly.

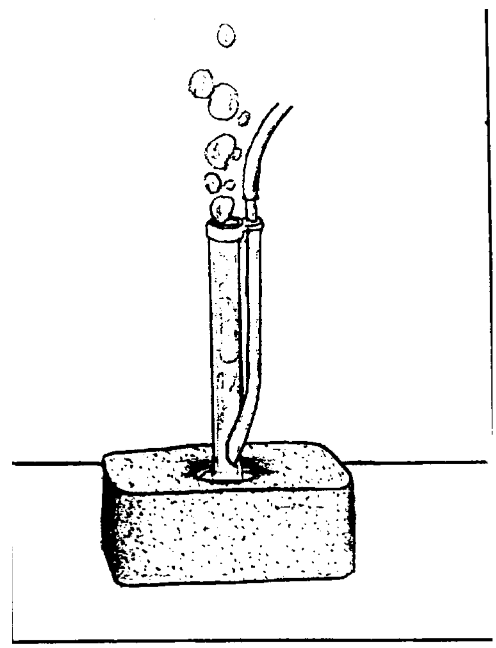


Fig. 2 - Typical sponge filter

An outside power filter is used on all tanks except those containing fry (Fig. 3). As the name implies, these are powerful filters capable of filtering an aquariums capacity several times each hour. This style of filter uses a siphon to remove water from the tank where it is run through a filter box and pumped back to the tank. The filtering media is usually a synthetic fiber that can be rinsed and re-used. Also, activated charcoal or carbon can be placed in the filter box to remove harmful gas buildups. Most new power filters now utilize filter cartridges consisting of a fiber barrier with carbon already added.

Cartridges should be rinsed clean with hot water twice weekly or as needed. On extremely crowded tanks, cartridges may need to be cleaned daily. Cartridges should be discarded and replaced after two months of use.

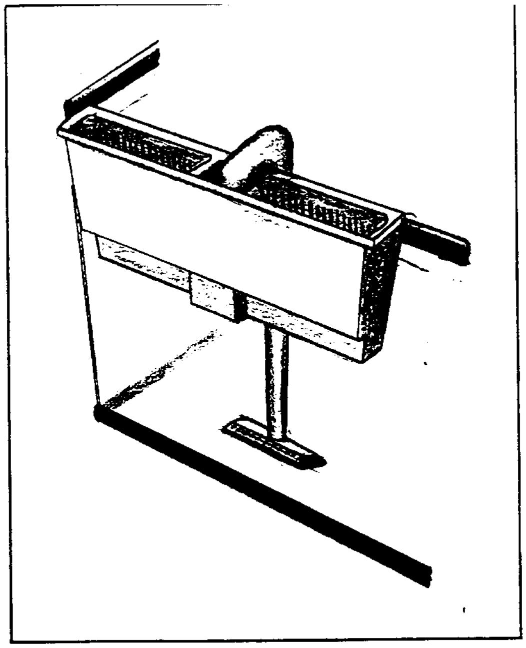


Fig. 3 - Power filter

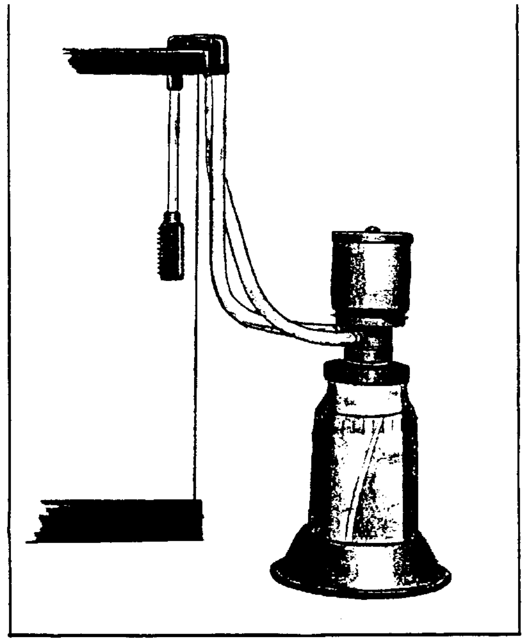


Fig. 4 - Canister filter

Another useful filtering device is the canister filter (Fig. 4). There are many models and most utilize diatomaceous earth as the filter media. These are auxiliary filters only and are capable of filtering down to .08 microns. These filters can actually remove parasites such *as Ichthyopthirius* from an aquarium. They should be run on each aquarium for 24 hours every two weeks as prophylaxis. Due to the high velocity flows discharged from these filters, they will stress the fish if used for more than 24 hours at a time. Most canister filters can be used in the presence of medications as long as the filter contains no charcoal or carbon.

LIGHTING

Individual aquarium lights are only needed on the spawning tanks. Wild fathead minnows spawn in late spring and summer so a photoperiod of 16 hours light to 8 hours dark must be maintained for maximum egg production. The Fort Worth program uses a simple appliance timer set at 5:00 AM to 9:00 PM.

All other tanks need only be illuminated for maintenance, netting fish and other operations. In fact, too much light on these other tanks encourages excessive algae growth causing extra maintenance time.

OTHER ACCESSORIES and SUPPLIES

There are many other accessories needed to complete the set-up. **Thermometers** for each tank are an absolute must as temperature control is critical in all operations. Spawning tanks are kept at 25C as well as the eggs. Rearing tanks can be left unheated in ambient laboratory temperatures of 20C to 27C. Avoid sudden temperature fluctuations in all aquariums.

A selection of **nets** is also necessary. Regardless of size, all net bags should be deep with rounded comers. Net sizes from 6" to 12" with a medium to coarse mesh are the most useful. Larger mesh grades allow the net to be pulled through the water with much less resistance than the finer grades. A few fine mesh nets should be purchased though, for the handling ofjuvenile specimens.

Two types of **medications** should always be kept on hand. A broad spectrum antibiotic will be needed to treat common bacterial infections and for prophylactic treatment of new fish introduced to the laboratory. Luckily, well kept minnows don't often fall prey to bacterial infections. The most frequent diseases encountered usually involve parasitic *protozoans. Ichthyophthiriasis and Oodinium,* conunonly called ich and velvet respectively, are frequent aquarium parasites yet both are treated easily by a number of medications available at any pet shop.

Fish foods to be considered in the initial purchase include dry food and frozen food. A quality flake type tropical fish food is usually the staple diet of most rninnows. This is supplemented with frozen bfine shrimp, *Artemia.* Live foods such as tubiflex worms, meal worms and small crustaceans are relished by the minnows but should be lin-dted as diseases are often introduced into aquafiums through the use of live foods. Freeze dfied foods can be very high in nutfitional value but are not used much in commercial programs due to their expense.

Chemicals used for conditioning the water are also needed. Many cities are adding chloramines to the drinking water and many commercial chlofine removers are ineffective at removing chloramines. There are many excellent water conditioners available from pet shops that not only remove chlofine/chloramines but also remove heavy metals, endotoxins, pheromones and a host of other toxins. Again, the manager of a well established pet shop can recommend the product best suited for a particular area.

Heaters may or may not be needed depending upon the facility set up. If ambient laboratory temperatures stay around 25C $\pm$ 5C, then heaters will probably not be needed. If heaters are needed, it is extremely important to match the heater wattage to the aquarium gallonage. A heater too small for the aquarium being heated will often malfunction resulting in the loss of the whole tank. The formula for matching heaters to aquariums is 50 watts of heating output per 10 gallons of water. Example; the 40 gallon breeder aquarium requires a 200 watt heater. Follow manufacturers instructions precisely with all heaters and monitor temperature in heated tanks at least twice daily.

An assortment of **plastic bags** will be needed for transferring and transporting minnows. These are available from aquarium dealers and container distributors in inexpensive, bulk form. The Fort Worth program uses 8" X 15" bags that are 2.0 n-d thick. These will hold one dozen minnows for six hours if the bag is oxygenated and kept cool. Larger bags will be needed to transport larger numbers of rninnows. A large ice chest lined with plastic garbage bags will hold three-hundred minnows for one hour. If the bag is oxygenated and tightly sealed, three-hundred minnows can be kept for two to four hours. If possible, do not feed rninnows for twelve hours before transport. Well fed minnows VAII excrete wastes in the transport bag which can foul the water within minutes.

A selection of **books** should complete the initial purchase. A general aquarium book will cover the basics of maintenance. A good fish disease book is invaluable and an absolute must unless the person in charge of the program is thoroughly familiar vath aquarium diseases. There are also a few publications that deal with the care and propagation of native fish. These are very useful as they deal with temperature, water quality parameters and other special needs of native fish.

The fathead minnow is one of the most studied fishes in history and there is much information available in the science literature. A short bibliography is included in this guide which lists several of these publications. The USEPA also has a videotape series which is included in the bibliography.

WATER SOURCES

Any toxicity free water can be used for culturing fathead minnows. It should be from a consistent source to assure constant water quality throughout the entire program.

City tap water is the most common water source in the urban environment. However, it must be treated before being used. Sodium thiosulphate is used to remove chlorine and stock solutions are available through pet shops, tropical fish stores and wholesale suppliers. Most of the newer commercial water conditioners will also remove chloramines, now common in many municipal water supplies.

Untreated well water is preferred by many fathead laboratories. Before using water from artesian sources, it should be tested for metals, pesticides or other possible groundwater contaminants.

Surface waters from lakes and streams can also be used but must be sterilized to eliminate any possible pathogens. The canister filter described in the filter section can be used for parasite elimination but an ultraviolet sterilizer is needed to kill unwanted bacteria.

Distilled water can be used but essential salts must be added. For each ten gallons of **distilled** water added to the aquarium, the following should be added;

21g common salt
7g potassium sulfate
7g magnesium sulfate

Do not use rainwater as it often collects airbome pollutants (including acid pH) which can be extremely toxic to fish in the lab.

AQUARIUM SET UP and CLEANING

Before assembly, all aquarium components should be thoroughly cleaned. The typical cleaning procedure is to rinse with fresh water, scrub with sodium bicarbonate or salt, then triple rinse with fresh water. Never use soaps or detergents on any aquarium equipment. Vinegar or a weak acetic acid solution may be used to remove light mineral deposits or waterlines from glass. Care must be taken when using any cleaning solutions that could affect the fish. After cleaning an empty aquarium with acetic acid, the tank should be triple rinsed with fresh water, scrubbed with sodium bicarbonate to neutralize any remaining acid residue and then triple rinsed again.

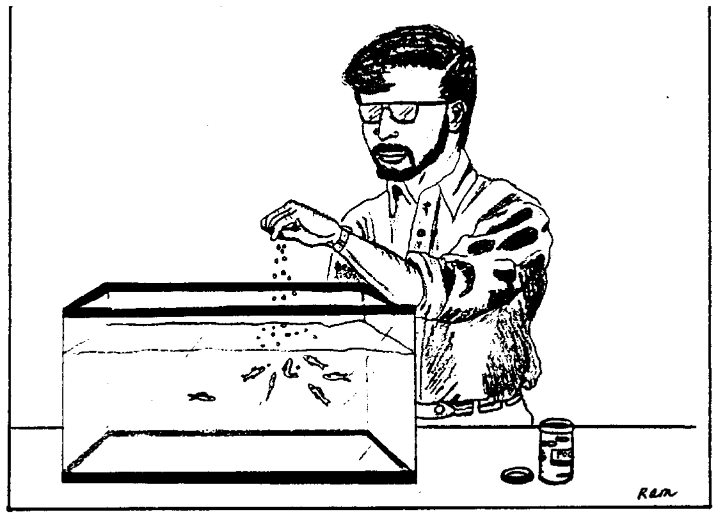
Glass tops, filters and anything else that comes in contact with the water should be cleaned in the same manner and a regular cleaning/maintenance routine should be planned for all equipment. This routine could be weekly, monthly or yearly depending upon the mineral content of the water.

Once cleaned, the aquariums can be placed in their permanent location and filled. Each aquarium should have its own air pump, air driven filter, airstone and outside power filter which can now be assembled and activated as per their individual instructions. Chlorine removal and other needed water treatments should be performed at this time. Tank covers, lights, thermometers and heaters should be assembled and activated last.

All aquariums should be allowed to run for at least a week before the addition of any fish, especially if city tap water is being used. Tap water is often under great pressure which forces an excess of unwanted dissolved gasses into the water. One week of aeration and carbon filtration through outside power filter will remove these gases.

When assembling the aquarium components, follow all manufacturers instructions carefully. if possible, keep all electrical components, such as air pumps, in areas safe from water spills or unexpected aquarium breakage.

CARE OF THE MINNOWS



TEMPERATURE REQUIREMENTS

As mentioned before, water temperature control is critical in a fathead breeding laboratory. All aquariums should be kept at 20C to 27C. The prime consideration is to maintain a constant temperature as sudden variations of more than 20C can be fatal to the test organisms. Spawning tanks should be maintained at 25C for optimum egg production. In rearing tanks, temperatures in the higher ranges of the fatheads tolerance range are preferred as the juveniles will grow faster than those held at cooler temperatures.

DISSOLVED OXYGEN

A dissolved oxygen level of at least 8.0 mg/L must be maintained in all tanks or growth rates will be affected dramatically. Maintaining this DO level is not a problem if airstones are properly operated and maintained as they will keep a tank near the 100% saturation level. All airstones should be periodically scraped clean as needed and replaced when cleaning will no longer restore the original air flow.

ACCLIMATION

Once the tanks have all been set-up and aged, the minnows may be introduced. Typically, small fish such as minnows are bought or collected in large numbers and bagged in lots of 100 to 500 fish per plastic bag. When introducing a large bag of fish to an aquarium, the bag should be floated for 30 minutes to allow the bag temperature to stabilize. During this stabilization period, every 5 minutes, add 500 ml of aquarium water to the bag so the fish can acclimate to the new water chemistry. If the minnows show signs of distress before they are completely acclimated, place an airstone into the bag. Once acclimation is complete, do not dump the contents of the bag into the aquarium. Instead, gently net the fish out and discard the water from the bag.

FEEDING

Three types of fish food are used in the Fort Worth program. These are; dry flake food, frozen brine shrimp and freshly hatched brine shrimp.

All fish are fed twice a day. Those over 30 days old are given all the frozen brine shrimp or commercial flake food they can eat within a fifteen minute period. On weekends they are fed once a day. Active spawners are often given several small supplemental feedings throughout the day to help keep them in peak condition. The usual routine is to give the fish two meals of flake food to every three meals of frozen brine shrimp. Algae growth in several of the Fort Worth aquariums is encouraged because it provides a good dietary supplement. Siphon all uneaten food from the aquariums within one-half an hour of the feeding.

All freshly hatched fry should be fed baby brine shrimp at least twice a day. In an active breeding program with hundreds of fatheads hatching daily, brine shrimp cultures must be started daily as shrimp more than 24 hours old are too large for the freshly hatched fatheads to ingest.

Hatching brine shrimp is a relatively easy task. Dried *Artemia salina* cysts are added to salt water and kept under constant, heavy aeration until hatched. Hatching occurs within 24 hours at 25C to 30C. A salinity medium is prepared with rock salt. The actual concentration is not critical as the shrimp will hatch in a wide range of concentrations. Typically, shrimp will hatch quicker in a lower concentration but they will live longer in a higher concentration. The simplest method is to use four teaspoons of rock salt per gallon of water. Aerate profusely and after the salt has dissolved, add a couple of drops of chlorine remover and 1/4 to 1/2 teaspoon of eggs.

Standard one gallon bottles may be used for hatching shrimp though conical shaped containers make collecting the hatched shrimp much easier. San Francisco Bay Brand, Inc., 8239 Enterprise Dr., Newark, California 94560 is the only manufacturer offering specialized brine shrimp hatcheries at the time of this writing. To collect the hatched shrimp, remove the aerator and allow time for the shrimp to settle. The live shrimp will form an orange layer near the bottom of the vessel. Unhatched cysts will form a thin brown layer below them and the hatched cyst pieces will float to the top. Siphon the orange layer into a suitably sized sieve, rinse with fresh water to remove excess salts and they are ready to feed to the fry.

Live Artemia will only live in the aquariums for 2 to 4 hours so do not feed more than the fry can consume in this period. However, some overfeeding is inevitable until a feel for feeding the fry is achieved so be alert to dead shrimp collecting on the bottom and siphon accordingly. The object is to keep the fishes stomachs constantly round and bulging without fouling the tank.

DISEASES

Disease outbreaks in properly maintained systems are rare. However, some disease is inevitable during the course of a study and the program manager should become familiar with the basic aquarium diseases and their treatment.

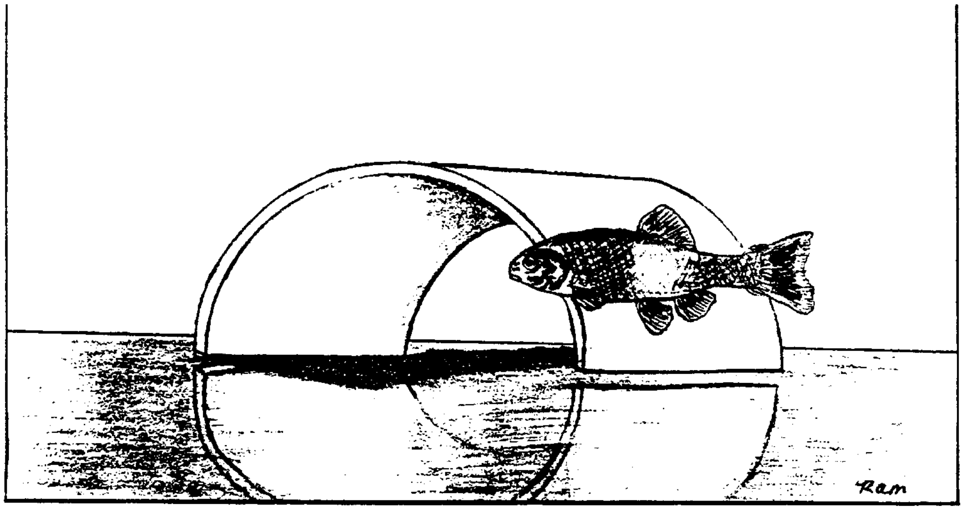
Bacterial infections are usually the most common diseases encountered in a minnow raising operation. V&le primary infections do sometimes occur, secondary infections to weak or injured fish are encountered more frequently. Fin and Tail Rot, Mouth Rot and Cotton Wool Disease are the three infections encountered the most and all can be effectively treated with penicillin or erythromycin derivatives available through the aquarium industry. All bacterial diseases are much easier to cure if the infection is discovered during the early stages. Any filter containing charcoal or carbon should be disconnected when treating a tank with antibiotics as the carbon will render the antibiotic ineffective. Antibiotics can interrupt nitrification also.

Parasitic infections usually occur when the fish are stressed by environmental changes such as rapid temperature drops. Also, parasites are frequently introduced to the laboratory with new fish or live foods. Parasites are easily eliminated by any number of effective medications available through the aquafium industry. Carbon filtration can be used with most parasite medications.

At least 25% of the tanks water should be changed daily on all tanks being medicated. Carefully match water temperatures and make water changes before the daily treatment. Follow the directions carefully when using any medication and take extra precautions to avoid cross contamination of the healthy tanks with the medicated tank. Disinfect all equipment before using on another tank and wash hands and arms thoroughly after working with an infected tank.

The biggest decision concerning medications is when to use them. If only a few fish are infected, it is usually better to destroy the infected fish, make a water change and then treat the tank. Only one or two days of treatment should then be needed. If you actually try to cure the infected specimens, treatment can last 7 to 14 days. As mentioned before, a <u>good</u> aquarium disease book is an invaluable addition to the laboratory.

PROPAGATION



SPAWNING TANKS

The number of sexually mature fatheads per spawning tank is dependent upon the size of the tank. Many labs use 20 gallon aquariums (24"X12"X1611) with 2 males and 5 to 8 females per tank. The Fort Worth program uses 10 gallon aquariums with 1 male and 2 to 4 females per tank.

Carefully check the spawning tanks daily for extra males. Sexually immature males are easily confused with females and must be removed immediately upon recognition. If not removed, the extra males will harass the mature male and eat eggs. Spawning males should be replaced after one year of breeding, or earlier, if egg production starts to drop.

Females contain eggs in all stages of development and will continue to spawn until the entire egg compliment is exhausted. Replace any spawning female once egg production starts to drop.

Fatheads lay adhesive eggs and prefer to deposit them on the underside of hard surfaces. A 6" long piece of 4" diameter PVC, cut in half, works well. Two spawning tiles are used for each male in the tank. If a larger spawning tank with two males is used, separate the spawning tiles as much as possible. Fathead males make up distinctive territories and they will aggressively protect their individual territories. For maximum egg production, it is important that these territories not overlap.

SPAWNING

Fathead minnows mature in 90 to 120 days. Sexing the minnows is not an easy task until they begin showing signs of sexual maturity. At this time, males are generally larger and darker in color. They develop enlarged heads and exhibit nuptial tubercles on the snout. Females are generally smaller than males of the same age, have a tapered snout and exhibit an ovipositor.

Spawning tanks should always be kept at 25C and the rainnows generaly begin spawning in the early moming hours. The male cleans the underside of the spawning tile and then draws an egg laden female underneath. As she releases the buoyant eggs, they stick to the underside of the tile and each other. Fertilization by the male is extemal. An average spawn will produce 100 to 200 eggs and females in excellent condition can spawn every 2 days although every 4 to 5 days is more typical.

Once spawning is complete, the male takes over and drives the female away from the spawning tile. He will clean and protect the eggs until they hatch. Unfortunately, not all males are good parents and it is best to remove the eggs as soon as spawning is completed.

In many fathead laboratories, the eggs are removed from the spawning tiles and placed in special hatching trays. This is the recommended procedure if embryo-larval and larval tests are to be performed. In this study though, only the adults are to be utilized as stream sentinels and accurate tracking of numbers of eggs, embryos and larvae is not necessary. This being the case, the eggs need not be removed from the spawning tiles.

Place the entire spawning tile in a glass container containing water from the spawning tank. Take care not to transfer uneaten food, algae or other debris into the hatchery vessel. Filter the water if needed. Add 20 drops of a 1% methelyne blue solution to each gallon of water in the hatchery container. This will lirnit fungal and bacterial infections in the container. Aerate the container vigorously and maintain a constant temperature of 25C. Due to the small size of most hatchery vessels, it may be easier to place the hatchery container in a water bath and heat the bath water.

Check the tfles daily and remove any dead or diseased eggs. Non-viable eggs are easily identified as they tum white and opaque. If more than 50% of the eggs on a tile die, discard the entire batch. These egg checks should be performed quickly to avoid desiccation and eggs in their later stages of development are mel fragile and should be handled accordingly.

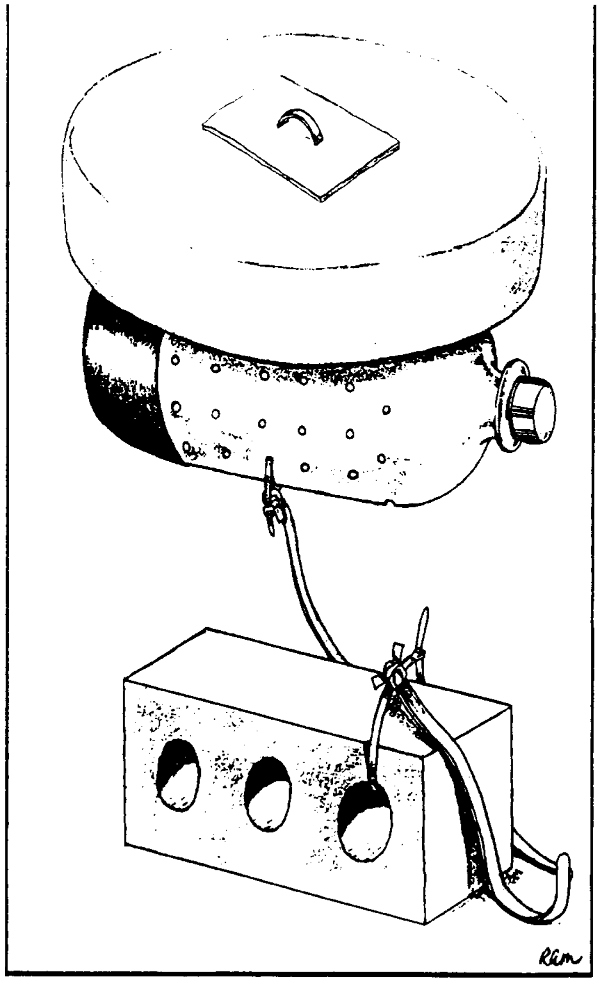
At 25C, hatching occurs in 4 to 5 days. Lower temperatures can delay hatching from 2 to 4 days while higher temperatures encourage fungal and bacterial growth.

Once the eggs have hatched, drain 3/4 of the water from the hatchery container and, over a one hour period, gradually refill it with water from the aquarium the fiy are to be placed in. Once acclimated, the hatching container can be placed in the rearing aquarium and gently poured in.

Used tiles should be scrubbed clean and stefilized in a weak chlofine solution before reuse. Tfiple rinse with fresh water, neutralize with chlofine remover and tfiple finse again.

All fry are fed freshly hatched brine shrimp nauplii twice daily until they are 30 days old. During their first few days, they require very little food and special attention must be given to avoid overfeeding and the subsequent fouling of the rearing tank. As they grow, so does their demand for food and the amount and size of shrimp may be increased proportionally. If all live shrimp is consumed within 30 minutes, increase the amount of shrimp, but not to the point that it is not all consumed before it dies.

After 30 days, the fry are moved to larger rearing tanks and fed frozen adult brine shrimp and flake foods. They should be large enough to utilize as stream sentinels within 90 to 120 days if filters have been kept clean and, most importantly, <u>at least 25% of the water in each tank has been changed weekly</u>.



STREAM SENTINEL STATIONS

In 1985, the City of Fort Worth began a series of intensive storm drain investigations. Due to the transient nature of most ston-n drain pollutants and the overall size and scope of the Fort Worth MS4, investigators were rarely able to be on scene when the pollutants were actually present. It became obvious that something was needed to document these events.

During the same period, the Fort Worth Program had developed a " Fish-In-A-Bottle " unit to assess stream recovery after a fish kill. This simple unit was then cross utilized and developed as the stream sentinel station now used to document toxic events in storrn drain systems 24 hours a day.

Only a few common supplies are needed to construct a sentinel station. A two liter soft drink bottle with cap is the main part of the unit. A float is attached along with an anchor.

The bottles used in the Fort Worth Program are new. They were purchased from a local container manufacturer for $60.00 per 396 bottles. Used bottles may be used for small programs needing only a few units. This could be one recycling project which would directly benefit your water quality monitoring program. All used bottles must be thoroughly cleansed with hot water and triple rinsed with distilled/deioniozed water.

Ventilation holes may be drilled in the bottles with an electric drill. However, it is easier and faster to melt the holes with a soldering iron. Several soldering irons can be used in series to melt multiple holes at once (Fig. 5). With this set-up, a complete bottle can be ventilated in less than a minute.

The floats used in the initial stages of the Fort Worth Program were actually styrofoam packing sleeves salvaged from containers used for shipping light fixtures. As the program expanded to include over one-hundred storm drain systems, a local styrofoam manufacturer was contracted to construct 100 disks, 2" thick by 12" in diameter. Cost was $90.00 for 300 disks. The float serves a dual purpose of suspending the station near the surface film while also providing shade for the minnows. For added strength, a section of heavy plastic needs to be mounted on top of the styrofoam disk. This VAII prevent the unit from pulling apart during high flows in the storm drain system. Also, this styrofoam surface provides an area where a message and phone number (public reporting) can be painted.

A brick with holes in it serves well as an anchor. For systems that experience extremely high flow velocities, multiple bricks can be fastened together. No bricks were purchased for the Fort Worth Program. Instead, usable bricks were salvaged from construction dump sites.

Anchors may be connected to the bottles by rope or wire. Trot-line rubberband line can be used as an anchor line in some systems. The rubberband line stretches as the water level rises, keeping the sentinel station on the surface. However, this elastic connector will break in high flows and a stronger connector and extra anchoring should be added in preparing for large storm events.

Fifteen inch tie-wraps are used to fasten all the components together. Detailed instructions on assembling a stream sentinal unit are presented in Part 2 of of the video tape series included in Section 4 of this document.

USES

Sentinel stations are ideal for long or short term studies. In the Fort Worth Program, three sites were monitored daily over a one year pefiod. Dead **fish** were removed and replaced dufing the daily checks and all fish, regardless of health, were replaced every two weeks.

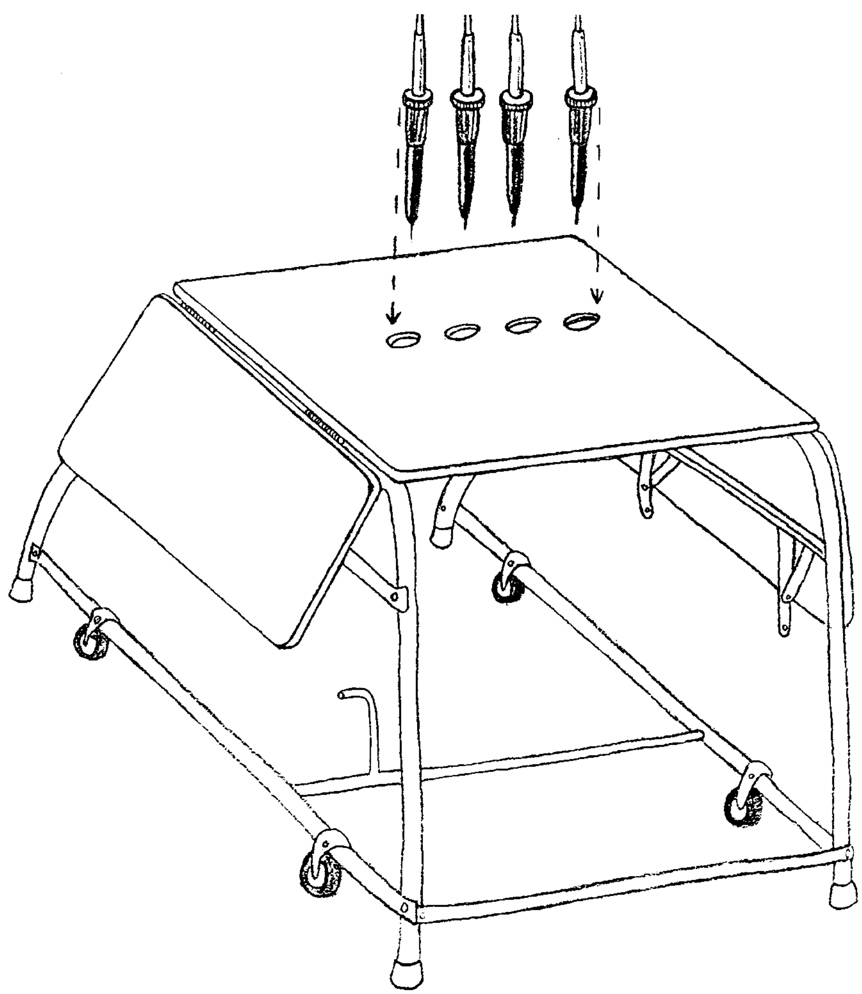


Fig. 6 - Set-up for using
multiple soldering irons.
Space irons 2" apart.

One-hundred additional sites were tested on a short term basis. These additional sites were monitored for one week with field checks performed once per day. AH surviving fish were released at the end of the week.

Several sites with previously identified, sporadic, toxic flows were tested for an eight week period to determine possible illicit flow trends. These sites were visited at least once per day and often twice to determine when these toxic events were occurring. Dead fish were replaced as needed during each visit with all fish being replaced after two weeks.

As described above, all fish involved in longer term studies are replaced after two weeks in the sentinel station. As fish are netted, transported and placed in the bottles, minor damage to scales, fins, gills and other organs can easily occur. Such insignificant damage rarely kills the fish quickly but often leads to bacterial infections. Obviously, these infections can not be treated while the fish are in the field and an injured fish will eventually succumb. Also, fish in sentinel stations can not feed well and they are subject to injury from strong currents. In short, any fish that dies in the bottle *after* two weeks may have died from influences not related to toxic flows.

Before releasing any minnows into the wild, get permission to do so from the State Game and Fish Department. All of these agencies have restrictions on the stocking of fish in public and pfivate waters and the introduction of a prolific fish like the fathead could severely impact other aquatic species in a sensitive or protected ecosystem.

SITE EVALUATION AND ASSESSMENT

Any body of water large enough to float a sentinel station can be monitored. The only sites not considered are those too dangerous to reach. Safety of the field staff is the most important consideration in determining whether a site is suitable for monitoring.

Try to determine if the site will have problems before starting to monitor. Will the water be too high to safely monitor during storm events? Check the waterline in the outfall pipe or look for stream bank erosion to determine maximum water levels. Will the sentinel station be vandalized? Numerous human footprints, empty beverage containers, bait containers and the like all indicate a high use area and probable vandalism.

However, if there are houses or businesses next to the monitor site, it helps to notify the residents as to what you are doing and how long you are monitoring. Experience has shown that most people are very interested in keeping the creek next door clean and they will tend to keep an eye on the site. Many times, these interested parties will visit the field staff when they arrive and report any unusual occurrences or observations of pollutant flows.

All field staff should become thoroughly familiar with the monitor site from the very beginning. Walk the waterway and make note of any animals or animal tracks around the stream. Get in the water and root through the vegetation and sediments to determine what aquatic insects are present. Are there any fish? Note the color and clarity of the water. Are there traces of sewage, oil sheens or other signs of pollution? The point is to know the normal condition of the site so problems can be spotted immediately.

Make note of any differences in a daily joumal, no matter how minor they may seem at the time. Over the course of the study, trends and pattems often emerge which can help tremendously in determining the sources of toxic flows.

TRANSPORT OF FISH TO THE BIOMONITORING SITE

FiB an 8" X 15" (or similar size) plastic bag to one third capacity with water from the aquarium containing the fish to be transported. Carefully net six specimens and place them directly in the bag. Any specimens bruised by the net frame should be discarded as well as any that jump out of the net or are dropped. Also, it should be noted that only healthy fish should be netted. Specimens with damaged scales and fins or those showing swimming irregularities should be discarded or placed in an isolated aquarium for treatment.

Before continuing, there are a few rules that apply to nets and the netting of fish that should be discussed. First and foremost, a net is used one time in one aquarium only. It must then be sterilized before re-use. Nets can be stored in a one gallon bucket of water with one-half teaspoon of potassium permanganate added. The sterilization solution should be rinsed from the net before use. Also, fish should never be placed in dry nets and if the fish must be handled directly, wet hands thoroughly first.

After six suitable specimens have been bagged, inflate the bag to full capacity with compressed air and seal tightly with a rubber band.Compressed air must be from a non-toxic source such as bottled breathing air. An aquarium air pump may also be dedicated for inflation of the fish bags. Place the sealed bag/bags in an ice chest and the specimens are ready for transport to the stream.

Upon arrival at the biomonitoring site, take the temperature of the fish bag and the stream. If the temperatures differ by more than 10F, add 200 ml of stream water to the bag every five minutes until temperature equilibrium occurs. Once this is reached, continue to add 50 ml of stream water to the bag every 30 seconds until the bag is 25% aquarium water and 75% stream water. The fish can now be transferred to the stream sentinel station.

If the stream and the **fish** bag are the same temperature or within 50F, simply add 50 ml of stream water to the bag every 30 seconds until the bag is 25% aquarium water and 75% stream water.

To place the specimens in the stream sentinel station, fill the undrilled bottom half of the bottle with stream water. Place a wet funnel in the bottle mouth. Discard 90% of the water in the fish bag (after acclimation) and gently pour the remaining water containing the fish down the side of the funnel. Remove the funnel, screw the closure cap tightly onto the bottle and the unit is ready for instream placement.

RECORDING DATA

An example of the Data Sheet used in the Fort Worth Program is shown in Figure 6. This form may be used as is or modified for use in another program. On any biomonitoring data sheet, there must be an accurate site description so other staff can find the exact site. Spaces should be provided for basic information such as date, time of day and signatures of the monitoring staff Spaces should also be provided for site specific parameters such as temperature and dissolved oxygen to create a base profile of the waterway. And, of course, spaces should be provided to record the status of the fish.

The Fort Worth form has spaces for detailing information on the sentinel station. These were included to keep track of what happens to these units during field use. As of this writing, a total of 195 sentinel stations have been placed in the field. Overall, 78 units, approximately 32% of the total, were stolen or washed away in storm flows. Twelve (12) units were vandalized and the fish were removed from 2 units though these two units were not damaged.

CITY OF FORT WORTH ENVIRONMENTAL QUALITY SECTION
DATA SHEET FOR "FISH IN THE BOTTLE"

LOCATION/SITE DESCRIPTION__________ MAPSCO__________

ADDRESS__________ OUTFALL NAME__________

#1 STATUS CIRCLE ONE

INITAL STOCK, FOLLOWUP STOCK OR RECHECK
DATE______ TIME______AM/PM
HRS SINCE LAST RAIN______
HRS SINCE LAST VISIT______
TEMPERATURE (C) AIR______ WATER______
CONTAINER______ ADJUSTED______
INSTREAM DISSOLVED OXYGEN______
SPECIES STOCKED______
CIRCLE ONE: UNIT OK, VANDALIZED, MISSING
MORTALITY: ______# DEAD/______# STOCKED
ALL REPLACED YES OR NO
BOTTLE REPLACED YES OR NO
USE BACK FOR ADDITIONAL COMMENTS
ADDITIONAL TESTS:
MICROTOX______
USGS______
DRY WEATHER FIELD SCREEN______
OTHER______
SIGNATURE______

#2 STATUS CIRCLE ONE

INITAL STOCK, FOLLOWUP STOCK OR RECHECK
DATE______ TIME______AM/PM
HRS SINCE LAST RAIN______
HRS SINCE LAST VISIT______
TEMPERATURE (C) AIR______ WATER______
CONTAINER______ ADJUSTED______
INSTREAM DISSOLVED OXYGEN______
SPECIES STOCKED______
CIRCLE ONE: UNIT OK, VANDALIZED, MISSING
MORTALITY: ______# DEAD/______# STOCKED
ALL REPLACED YES OR NO
BOTTLE REPLACED YES OR NO
USE BACK FOR ADDITIONAL COMMENTS
ADDITIONAL TESTS:
MICROTOX______
USGS______
DRY WEATHER FIELD SCREEN______
OTHER______
SIGNATURE______

#3 STATUS CIRCLE ONE

INITAL STOCK, FOLLOWUP STOCK OR RECHECK
DATE______ TIME______AM/ PM
HRS SINCE LAST RAIN______
HRS SINCE LAST VISIT______
TEMPERATURE (C) AIR______ WATER______
CONTAINER______ ADJUSTED______
INSTREAM DISSOLVED OXYGEN______
SPECIES STOCKED______
CIRCLE ONE: UNIT OK, VANDALIZED, MISSING
MORTALITY: ______# DEAD/______# STOCKED
ALL REPLACED YES OR NO
BOTTLE REPLACED YES OR NO
USE BACK FOR ADDITIONAL COMMENTS
ADDITIONAL TESTS:
MICROTOX______
USGS______
DRY WEATHER FIELD SCREEN______
OTHER______
SIGNATURE______

#4 STATUS CIRCLE ONE

INITAL STOCK, FOLLOWUP STOCK OR RECHECK
DATE______ TIME______AM/PM
HRS SINCE LAST RAIN______
HRS SINCE LAST VISIT______
TEMPERATURE (C) AIR______ WATER______
CONTAINER______ ADJUSTED______
INSTREAM DISSOLVED OXYGEN______
SPECIES STOCKED______
CIRCLE ONE: UNIT OK, VANDALIZED, MISSING
MORTALITY: ______# DEAD/______# STOCKED
ALL REPLACED YES OR NO
BOTTLE REPLACED YES OR NO
USE BACK FOR ADDITIONAL COMMENTS
ADDITIONAL TESTS:
MICROTOX______
USGS______
DRY WEATHER FIELD SCREEN______
OTHER______
SIGNATURE______

#5 STATUS CIRCLE ONE

INITAL STOCK, FOLLOWUP STOCK OR RECHECK
DATE______ TIME______AM/PM
HRS SINCE LAST RAIN______
HRS SINCE LAST VISIT______
TEMPERATAURE (C) AIR______ WATER______
CONTAINER______ ADJUSTED______
INSTREAM DISSOLVED OXYGEN______
SPECIES STOCKED______
CIRCLE ONE: UNIT OK, VANDALIZED, MISSING
MORTALITY: ______# DEAD/______# STOCKED
ALL REPLACED YES OR NO
BOTTLE REPLACED YES OR NO
USE BACK FOR ADDITIONAL COMMENTS
ADDITIONAL TESTS:
MICROTOX______
USGS______
DRY WEATHER FIELD SCREEN______
OTHER______
SIGNATURE______

#6 STATUS CIRCLE ONE

INITAL STOCK, FOLLOWUP STOCK OR RECHECK
DATE______ TIME______AM/ PM
HRS SINCE LAST RAIN______
HRS SINCE LAST VISIT______
TEMPERATURE (C) AIR______ WATER______
CONTAINER______ ADJUSTED______
INSTREAM DISSOLVED OXYGEN______
SPECIES STOCKED______
CIRCLE ONE: UNIT OK, VANDALIZED, MISSING
MORTALITY: ______# DEAD/______# STOCKED
ALL REPLACED YES OR NO
BOTTLE REPLACED YES OR NO
USE BACK FOR ADDITIONAL COMMENTS
ADDITIONAL TESTS:
MICROTOX______
USGS______
DRY WEATHER FIELD SCREEN______
OTHER______
SIGNATURE______

Fig. 6 - Data sheet

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Video Tapes

EPA. 1989. Culturing Fathead Nfinnows *(Pimephalespromelas)*. EPA/505/8-89/002b. Supplemental Report for Video Training Tape. Washington, DC.
This 17: 1 0 minute video tape and supplemental report was produced by the EPA's Office of Water Enforcement and Permits. It is available through the National AudioVisual Center, Capital Heights, Maryland 20743.

City of Fort Worth, 1989. Storm Drain System Pollution Control. This video was produced with Ford Foundation funds and provides an overview of Fort Worth's earlier activities including the fish in the bottle. A limited number of copies are available upon request. Fort Worth Department of Environmental Management, 5000 M.L.K. Freeway, Fort Worth, Texas 76119. Duplication and mailing fees may be applicable.